Acidic Xylanase(ACX) Activity Assay Kit

Product Code: 112889

Acidic Xylanase (ACX) Activity Assay Kit - Spectrophotometric Method

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Package size: 50T

Product Introduction

Xylanase (EC 3.2.1.8)

Xylanase is mainly produced by microorganisms and catalyzes the hydrolysis of xylan. It is also known as pentosanase or hemicellulase. It can break down raw material cell walls and β-glucan, reduce material viscosity in brewing, promote the release of active substances, and reduce non-starch polysaccharides in feed, thereby improving nutrient absorption and utilization.

ACX is generally isolated from acid-tolerant fungi, bacteria, and some molds. It degrades xylan into reducing oligosaccharides and monosaccharides. These products react with 3,5-dinitrosalicylic acid to produce color, with a characteristic absorption peak at 540 nm. The color intensity is proportional to the amount of reducing sugars produced by enzymatic hydrolysis, so ACX activity can be calculated by measuring the increase in absorbance at 540 nm.

Package Contents

Code	Item	Quantity
112889.1	Reagent I	1 bottle
112889.2	Reagent II	1 bottle
112889.3	Buffer	1 bottle
112889.m	Manual	1 copy

Quality Standards and Safety Instructions

Raw Material and Package Name	Quality Standard	Main Toxicity
Reagent I	-	-
Reagent II	-	-
Buffer	-	-

Transportation and Storage Conditions

Item	Condition
Transportation	Transport with ice packs.
Storage	Store at 2-8 °C, protected from light.
Shelf life	90 days

Product Instructions

1. Crude Enzyme Extract Preparation

1. Fermentation broth: Centrifuge the fermentation broth at 8000 g and 4 °C for 15 min. Collect the supernatant as the sample to be tested.
2. Enzyme dry powder: Weigh about 0.1 mg and add 1 mL buffer to dissolve for testing.

3. Tissue sample: Prepare according to a tissue mass (g) to extraction solution volume (mL) ratio of 1:5-10. It is recommended to weigh about 0.1 g tissue and add 1 mL buffer. Homogenize in an ice bath, then centrifuge at 8000 g and 4 °C for 10 min. Collect the supernatant for testing.

2. Assay Procedure

Set one control tube for each assay tube.

Component	Control Tube	Assay Tube
Sample (μL)	200	200
Buffer (μL)	300	300
Reagent I (μL)	200	

Mix well, incubate in a 50 °C water bath for 30 min, then immediately place in a boiling water bath for 10 min to inactivate. Do not let the cap pop open, to prevent water from entering and changing the reaction system.

Component	Control Tube	Assay Tube
Reagent I (μL)	200	
Reagent II (μL)	300	300

Mix well and develop color in a boiling water bath for 5 min. Do not let the cap pop open, to prevent water from entering and altering the reaction system. Use a 1 mL glass cuvette to measure absorbance at 540 nm.

Calculate $\Delta A = A_{assay\ tube} - A_{control\ tube}$.

3. Activity Calculation

Standard curve: $y = 2.5554x - 0.002$, $R^2 = 0.9983$

3.1 Calculation Based on Liquid Volume

Enzyme activity definition: Under 50 °C and pH 4.8 conditions, the amount of enzyme required per milliliter of liquid sample per minute to decompose xylan and produce 1 nmol reducing sugar is defined as one unit of acidic xylanase activity.

ACX activity (nmol/min/mL) = $(\Delta A + 0.002) \div 2.5554 \div 150 \div T \times$ dilution factor $\times 10^6 = 435 \times (\Delta A + 0.002)$

3.2 Calculation Based on Protein Concentration

Enzyme activity definition: Under 50 °C and pH 4.8 conditions, the amount of enzyme required per milligram of protein per minute to decompose xylan and produce 1 nmol reducing sugar is defined as one unit of acidic xylanase activity.

ACX activity (nmol/min/mg prot) = $(\Delta A + 0.002) \div 2.5554 \div 150 \div T \times$ dilution factor $\times 10^6 \div$ Cpr $= 435 \times (\Delta A + 0.002) \div$ Cpr

3.3 Calculation on a Fresh Weight Basis

Enzyme activity definition: Under 50 °C and pH 4.8 conditions, the amount of enzyme required per gram of sample per minute to decompose xylan and produce 1 nmol of reducing sugar is one activity unit of acidic xylanase.

ACX activity (nmol/min/g fresh weight) = $(\Delta A + 0.002) \div 2.5554 \div 150 \div T \times$ dilution factor $\times 10^6 \div W = 435 \times (\Delta A + 0.002) \div W$

150: molecular weight of xylose

T: reaction time, 30 min

Dilution factor = $V_{reaction\ total} \div V_{sample} = 1000\ \mu L \div 200\ \mu L = 5$

10^6: conversion factor, namely 1 mg/mL = 10^6ng/mL

Cpr: sample protein concentration, mg/mL

W: sample mass, g

Notes

1. Before formal determination, select 2-3 samples with large expected differences for pretesting.
2. Required instruments and supplies: balance, refrigerated centrifuge, constant-temperature water bath, visible spectrophotometer, 1 mL glass cuvette, and distilled water.
3. If Reagent I shows white flocculent or granular precipitates, heat at 60 °C to dissolve before use.
4. The absorbance change should be controlled within 0.01-0.8. Otherwise, increase the sample amount or dilute the sample. If dilution is used, adjust the dilution factor in the calculation formula accordingly.
5. Store the kit at 2-8 °C. Shelf life is 3 months. It is recommended to use it as soon as possible.